

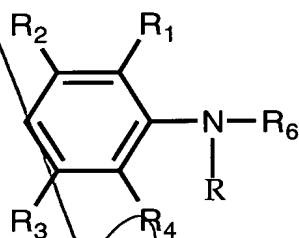
**WE CLAIM:**

1. An analytical element for the determination of acetaminophen in an aqueous fluid comprising a support having thereon at least one reagent layer and containing in said reagent layer:

(a) an arylacylamidase enzyme;

(b) an oxidizing agent selected from the group consisting of an enzyme and a ferricyanide capable of oxidatively coupling paraaminophenol to a coupling agent to form a color compound; and

(c) a water-soluble, color-forming, coupling agent of the general structure:



wherein R is a water-solubilizing group selected from  $-(CH_2)_nX$  where n is 1 to 5 and X is  $-SO_3M$  where M is hydrogen, an alkali metal, an alkaline earth metal or an ammonium ( $NH_4^+$ ) cation, or  $-N(R_7)_3^+Z^-$  where each R<sub>7</sub> is independently selected from alkyl of 1 to 4 carbon atoms; and Z is an acid anion; or X is  $(-OCH_2CH_2)_yOH$  where y is 2 to 5;

R<sub>1</sub> and R<sub>6</sub> are taken together to represent an ethylene, trimethylene, or tetramethylene group which forms a partially saturated ring;

R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are independently selected from hydrogen, alkyl of 1 to 4 carbon atoms, and alkoxy of 1 to 4 carbon atoms.

2. An analytical element of claim 1 for the determination of acetaminophen in an aqueous fluid comprising a support having thereon at least one reagent layer and containing in said reagent layer:

8. The element of ~~claim 2~~ further containing maleimide.

9. A multilayer analytical element of claim 1 for the determination of acetaminophen in an aqueous fluid comprising a support having thereon, in order from said support and in fluid contact:

(a) one or more layers having therein an arylacylamidase enzyme; a ferricyanide capable of oxidatively coupling paraaminophenol to a color-forming coupler to form a color compound; and a water-soluble, color-forming coupling agent as defined in claim 1; and

(b) a porous spreading layer.

10. The element of claim 3 wherein the ferricyanide is a ferricyanide salt of an alkali metal.

11. The element of claim 3 wherein the coupling agent is 1-(3-sulfopropyl)-1,2,3,4-tetrahydroquinoline.

12. The element of claim 3 further containing a buffer for maintaining the pH of the element in a range of about 6.5 to 8.5.

13. The element of claim 3 further containing maleimide.

14. A multilayer analytical element of claim 1 for the determination of acetaminophen in an aqueous fluid comprising a support having thereon, in order from said support a first and second reagent layer wherein:

the first reagent layer having therein 1-(3-sulfopropyl)-1,2,3,4-tetrahydroquinoline;

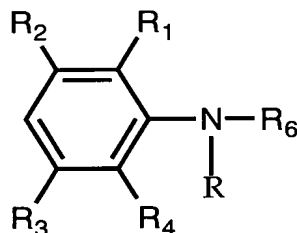
the second reagent layer having therein ascorbic acid oxidase or a ferricyanide salt and arylacylamidase; and  
a porous spreading layer.

15. The element of claim 14 further containing maleimide.

16. The element of claim 15 wherein the maleimide is in the spreading layer.

- (a) an arylacylamidase enzyme;
- (b) an oxidizing enzyme capable of oxidatively coupling paraaminophenol to a coupling agent to form a color compound; and

5 (c) a water-soluble, color-forming, coupling agent of the general structure:



10

wherein R is a water-solubilizing group selected from  $-(CH_2)_nX$  where n is 1 to 5 and X is  $-SO_3M$  where M is hydrogen, an alkali metal, an alkaline earth metal or an ammonium ( $NH_4^+$ ) cation, or  $-N(R_7)_3^+Z^-$  where each R<sub>7</sub> is independently selected from alkyl of 1 to 4 carbon atoms; and Z is an acid anion; or X is  $(-OCH_2CH_2)_yOH$  where y is 2 to 5;

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R<sub>1</sub> and R<sub>6</sub> are taken together to represent an ethylene, trimethylene, or tetramethylene group which forms a partially saturated ring;

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R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are independently selected from hydrogen, alkyl of 1 to 4 carbon atoms, and alkoxy of 1 to 4 carbon atoms.

3. An analytical of element of claim 1 for the determination of acetaminophen in an aqueous fluid comprising a support having thereon at least one reagent layer and containing in said reagent layer:

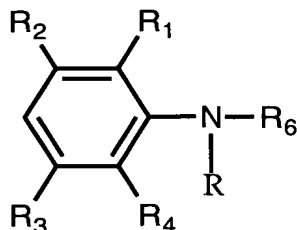
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(a) an arylacylamidase enzyme;

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(b) a ferricyanide capable of oxidatively coupling paraaminophenol to a coupling agent to form a color compound; and

(c) a water-soluble, color-forming, coupling agent of the general structure:



wherein R is a water-solubilizing group selected from  $-(CH_2)_nX$  where n is 1 to 5 and X is  $-SO_3M$  where M is hydrogen, an alkali metal, an alkaline earth metal or an ammonium ( $NH_4^+$ ) cation, or  $-N(R_7)_3^+Z^-$  where each R<sub>7</sub> is independently selected from alkyl of 1 to 4 carbon atoms; and Z is an acid anion; or X is  $(-OCH_2CH_2)_yOH$  where y is 2 to 5;

R<sub>1</sub> and R<sub>6</sub> are taken together to represent an ethylene, trimethylene, or tetramethylene group which forms a partially saturated ring;

R<sub>2</sub>, R<sub>3</sub>, and R<sub>4</sub> are independently selected from hydrogen, alkyl of 1 to 4 carbon atoms, and alkoxy of 1 to 4 carbon atoms.

4. A multilayer analytical element of claim 1 for the determination of acetaminophen in an aqueous fluid comprising a support having thereon, in order from said support and in fluid contact:

(a) one or more layers having therein an ~~an~~ ~~acyl~~ ~~amidase~~ ~~enzyme~~; an oxidizing enzyme capable of oxidatively coupling paraaminophenol to a color-forming coupler to form a color compound; and a water-soluble, color-forming coupling agent as defined in claim 1; and

(b) a porous spreading layer.

5. The element of claim 2 wherein the oxidizing enzyme is selected from the group consisting of ascorbic acid oxidase, lactase, and tyrosinase.

6. The element of claim 2 wherein the coupling agent is 1-(3-sulfopropyl)-1,2,3,4-tetrahydroquinoline.

7. The element of claim 2, further containing a buffer for maintaining the pH of the element in a range of about 6.5 to about 8.5.

17. A method for determining acetaminophen in an aqueous liquid comprising the steps of:

- a. contacting a sample of the aqueous liquid with the analytical element of claim 1, 2, 3, or 14; and
- b. correlating the amount of color compound formed to the concentration of acetaminophen in the fluid.